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Production of Healthy Brown Rice from Three Various Color Rice

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Abstract

This study was aimed at investigating the following: 1) Rice formula selections accepted by consumers; 2) levels of γ-oryzanol, α-tocopherol, total phenols and antioxidant activities of rice formulas accepted by consumers by using three strains of brown rice, namely, Khaw Dok Mali 105, Aromatic Black Rice and Fragrant Red Rice, respectively, to alter ratios in mixing four rice formulas. Sensory measurements were then taken to select rice formulas accepted by consumers with measurements of all γ-oryzanol, α-tocopherol, total phenol levels, antioxidant activities (before and after cooking), and measured GPx levels in rats under heat stress. According to the findings, Rice Formula 2 was the rice formula most widely accepted by tasters for tested properties. After cooking rice with heat, Rice Formula 2 (after cooking), had reduced γ-oryzanol, α-tocopherol, total phenol levels and antioxidant activities with statistical significance in every method. Furthermore, a temperature of 38±2°C was found capable of giving rats higher GPx while polyphenol substances from extracts of Rice Formula 2 at 500 mg/kg were found to have the effect of reducing GPx in rats. Based on this study, rice may be indicated as a naturally encountered antioxidant source. Polyphenol substances from rice extracts were found capable of reducing heat-related stress to the red blood cells of rats.

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Keywords: Healthy brown rice, Antioxidants, Glutathione peroxidase

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1. Introduction

Rice is a staple food consumed by over half of the world's population [1]. Rice grains usually have hard husks covering the grain. Rice used for consumption requires milling to separate rice husks from rice grain. The product is brown rice with rice germ and seed coat (bran) [2]. In addition to being a source of protein, carbohydrates, vitamins and minerals, rice is also abundant with plant nutrients for the human body [3] and [4]. Previous studies have reported rice as an abundant source of substances with biological effects, including γ-oryzanol, tocopherol, tocotrienol, phenolic compounds, phytic acid and phytosterol, etc., which have significant roles in promoting health and preventing a number of diseases while also promoting antioxidant activities [5]-[11]. It is commonly known that rice with black and red pigments tend to have better antioxidant capacity than rice with white pigment [12] and [13].

Heat-related stress is a significant problem for animals, especially in areas of the world with hot climates, because heat-related stress has impacts on animal growth rates, immunity and high mortality rates [14] and [15]. When the temperature is higher than 30°C, heat-related stress tends to manifest itself [16], causing biochemical and physiological activities related to increased oxidants in the oxygen group, which may prevent electrons from being transported via cell membranes [17] and may be a factor causing molecular changes encountered in DNA, protein, fat and other biomolecules [18]. Animals and humans undergo biochemical and physiological adaptations caused by heat-related stress in order to restore cell functions to normal [17]. Many studies have indicated that exposure to heat may cause animals and humans to experience oxidation stress [19] and [20]. Previous studies have reported phenolic substances to influence oxidation stress prevention [20] and [21] with key factors in preventing oxidation stress by oxidants in the oxygen group being enzymatic antioxidant systems including glutathione peroxidase, catalase and superoxide dismutase [22]. At this point, relationships between polyphenols from brown rice and glutathione peroxidase (GPx) have not been reported. The findings of this study will become basic data in using naturally encountered antioxidants with capacity to reduce heat-related stress.

Therefore, the objectives of this study were to select rice formulas accepted by consumers, examine total γ-oryzanol, α-tocopherol and total phenolic substance levels from the aforementioned rice formulas and study the antioxidant activities of rice formulas accepted by consumers *In Vitro*. The findings of the aforementioned study will become basic knowledge for producing healthy foods for humans as the world's weather grows warmer.

2. Materials and Methods

2.1. Rice Samples

This experiment used three strains of rice, namely, Khaw Dok Mali 105, Aromatic Black Rice and Fragrant Red Rice. All of the rice was planted in Selaphum, Roi Ed. Thailand, during the 2011 harvest season. Before analysis, the researcher milled the rice to separate the rice husks from brown rice by using a medium-sized brown rice milling machine belonging to the Science Program in Food Technology and Nutrition, Faculty of Technology, Mahasarakham University. The rice samples obtained were then stored in sealed black bags at a cool room temperature (4°C) for experimental use.

2.2. Experimental Design

2.2.1. Sensory Test Evaluation

Three strains of brown rice, namely, Khaw Dok Mali 105, Aromatic Black Rice and Fragrant Red Rice,

respectively, were used to alter ratios for mixing the following four rice formulas: Rice Formula 1 (2:2:2), Rice Formula 2 (1:2.5:2.5), Rice Formula 3 (1:3:2) and Rice Formula 4 (1.5:1.5:3). The rice was then quickly rinsed twice. A water-to-rice ratio of 4:1 (v/w) was used to cook all four prepared formulas by using electric rice cookers simultaneously. Sensory measurements were taken to select rice formulas accepted by consumers by using twenty tasters who were Mahasarakham University personnel aged thirty years and up. Taster scoring was based on a Hedonic scale scoring test by dividing score levels into a 9-point hedonic scale of preference as follows: 1 = Most Disliked, 2 = Very Disliked, 3 = Moderately Disliked, 4 = Mildly Disliked, 5 = Unable to Tell, 6 = Mildly Liked, 7 = Moderately Liked, 8 = Very Liked and 9 = Most Liked. During the tasting, the tasters sat in the tasting laboratory and were served samples of cooked rice from each formula, water and the questionnaire for testing the sensory acceptance of consumers [23].

2.2.2. Determination of Bioactive Compounds

- **Preparation of Crude Extract**: The test method was modified from [24] soaking powders of rice formulas accepted by consumers (before and after cooking) in 95%-methanol solvent to dissolve in distilled water at a ratio of 1:5 (w/v) for 24 hr. The mixture was then spun with a centrifuge machine at 2,500 rpm for 20 min and filtered with Whatman No.1 filtering paper. Next, the mixture was dried under vacuum conditions with a rotary evaporator at 45°C. The rough extracts obtained were used for further experimentation.
- **Determination of Total Phenolic Compounds**: TPC amounts were determined by the Folin-Ciocalteu method, an experimentation method modified from [24] and [25]. Rough extracts from rice at a volume of 200 µl were mixed with 1 ml of Folin-Ciocalteu reagents (diluted with disinfected distilled water at a ratio of 1:100) and 800 µl of a 10% Na2CO3 solution was added. Next, the disinfected distilled water was added to adjust the total volume to 5 ml. The mixture was blended and left to react for 2 hr before being measured for light absorption values at a wavelength of 760 nm. The standard antioxidant used to compare with the experiment results was Gallic acid.
- **Extraction and Determination of γ-oryzanol and α-tocopherol**: The experimentation method modified from [24] and [26] involved 1 g of soaking powder of rice formulas accepted by consumers (before and after cooking) in acetone at a ratio of 1:10 (w/v). The mixture was then spun with a centrifuge machine at maximum speed for 1 min and at 2,500 rpm for 20 min. Supernatants will be obtained after the solvent has been separated. Supernatants were then dehydrated under nitrogen.

Measurement of γ-oryzanol and α-tocopherol levels by the HPLC method was performed by dissolving rough extracts in the mobile phase and filtering by using a syringe-driven filter with a diameter of 0.45 µm. Analysis was performed using a RP-HPLC system machine (Shimadzu). 20 µl of the sample were injected through the security guard-column. Column size was 4.60 × 250 mm, 4 µl. Column temperature was controlled at 45°C. The mobile phase solvent, acetonitrile/methanol (25:75, v/v), was used at a flow rate of 1.5 ml/min. The wavelength was detected at 292 nm for measuring α-tocopherol levels and 325 nm for measuring γ-oryzanol levels.

2.2.3. Determination of Antioxidant Activity

2.2.3.1 In Vitro

2.2.3.1.1. Free-Radical Scavenging Activity on DPPH•

The DPPH method tested ability to scavenge DPPH• radicals. The experimentation method modified from [24] and [27] mixed 100 µl in a volume of rough extract from rice with 0.1 mM of concentrated DPPH• solution at a volume of 1.9 ml. The obtained mixture will be fermented in a dark space at room temperature

for 30 min before being measured for light absorption values at a wavelength of 517 nm. The control group consisted of the following: (1) standard BHA antioxidant (butylated hydroxyanisole) and (2) methanol. The measured values were used to calculate DPPH$^{\bullet}$ radical scavenging percentage from the equation [1 - (A_{517} of sample/ A_{517} of control)] × 100. IC_{50} (mg/ml) (concentration value of extracts capable of scavenging DPPH$^{\bullet}$ at 50%) was then calculated from the graph of relationships between percentage scavenging and extract amount.

2.2.3.1.2. Ferric Reducing Ability of Plasma (FRAP) Assay

FRAP is the method for testing ability to change Fe^{3+} to Fe^{2+} in which the reaction creates a dark blue color. This analysis can be performed by preparing a FRAP stock solution containing the following: (1) 300 mM concentrated acetate buffer at pH 3.6, (2) 10 mM concentrated TPTZ solution in 40 mM concentrated HCl and (3) 20 mM concentrated $FeCl_3 \bullet 6H_2O$ solution. The aforementioned stock solution was then fermented at 37°C before being used for analysis of antioxidant amounts. The experimentation method modified from [24] and [28] mixed 300 µl of rough extracts from rice with FRAP solution at a volume of 1.7 ml. The mixture obtained was then left to react at room temperature for 1 hr. Next, the mixture was measured for light absorption values at a wavelength of 593 nm. The standard antioxidant used to compare with the findings was $FeSO_4$ with a known concentration value. The analysis results were displayed in µmol $FeSO_4$/g fresh weight.

2.2.3.2. In Vivo

2.2.3.2.1. Animal Experiment

This research was conducted under the supervision of the committee on animal experimentation at Mahasarakham University, Mahasarakham, Thailand, according to Description No. 0001/2556 by using forty male Wistar rats aged six weeks (ten rats/group) weighing 200±50 g from the National Animal Laboratory Centre, Mahidol University, Salaya, Nakhon Pathom. Before the experiment, the rats were allowed to become accustomed to the environment for seven days with one rat living in one cage. The experimentation method was modified from [29] controlled environments as follows: Temperature of 23±2°C, relative humidity of 60% and ratio of time living in darkness:light (12:12 hr).

2.2.3.2.2. Preparation of Animal

The rats were randomly assigned to the four following groups: 1) The control group was given processed experimental rat food and water; 2) The first group was given processed experimental rat food, water and increased polyphenol from extracts of rice formulas accepted by consumers at 500 mg/kg; 3) The second group was given processed experimental rat food, water and were stimulated with stress by raising the temperature to 38±2°C for 4 hr/day and 4) The third group was given processed experimental rat food, water and were stimulated with stress by raising the temperature to 38±2°C for 4 hr/day with polyphenol from extracts of rice formulas accepted by consumers at 500 mg/kg. Blood samples were then collected at five intervals (Day 0, Day 1, Day 3, Day 7 and Day 14) to prepare red blood cells and measure GPx levels by using the CGP1-1KT Glutathione Peroxidase Cellular analysis set.

2.3. Statistical Analysis

Statistical data were analyzed by Analysis of Variance (ANOVA) with a completely randomized design. Selection of rice formulas accepted by consumers, all levels of γ-oryzanol, α-tocopherol and phenolic substances and antioxidant activities of rice formulas accepted by consumers *In Vitro* were analyzed by one-way ANOVA. The GPx levels in rats were analyzed by two-way ANOVA and were measured by comparing mean value differences in pairs with Scheffe's Test with statistical significance set at $p<0.05$.

3. Results and Discussion

3.1. Sensory Test Evaluation

Twenty tasters tasted four cooked rice formulas. The tasters had the highest mean preference for all tested properties in Rice Formula 2 (Khaw Dok Mali 105, Aromatic Black Rice and Fragrant Red Rice; 1:2.5:2.5) (Table 1). When the four formulas of cooked rice were compared, the tasters were found to have different preferences for Rice Formula 2 concerning color, scent and taste with statistical significance (p<0.05) with low preference for softness, possibly due to the taster factors involving age, gender and consumption taste together with the fact that the ratio of Khaw Dok Mali 105 was lower than that of Aromatic Black Rice and Fragrant Red Rice, which made the rice less soft than other rice formulas. Therefore, the most important consideration in selecting rice formulas was sensory consumer acceptance.

Table 1. Sensory consumer acceptance towards various properties by the 9-Hedonic Scale

Rice Formula	Color and Smell	Taste	Texture	Softness	Overall Acceptance
1 (2:2:2)	6.50±1.27[b]	7.10±1.66[a,b]	7.10±0.57[a]	7.20±1.23[a]	6.60±0.97[a]
2 (1:2.5:2.5)	8.40±0.52[a]	7.50±1.08[a]	7.40±0.84[a]	6.60±1.90[a]	7.60±1.35[a]
3 (1:3:2)	6.90±1.85[b]	7.10±1.20[a,b]	6.70±1.95[a]	6.70±1.34[a]	6.90±1.37[a]
4 (1.5:1.5:3)	6.60±1.58[b]	6.20±1.40[b]	7.20±1.40[a]	7.40±1.26[a]	7.20±1.14[a]

[a,b] Numbers marked with different letters in vertical order were statistically significant (p<0.05).

3.2. Bioactive Compounds

Rice Formula 2 (before cooking) contained all levels of γ-oryzanol, α-tocopherol and phenolic substances of 42.91 mg/100g, 0.30 µg/ml and 0.85 mg GAE/g, respectively, which were higher than 29.15 mg/100g, 0.24 µg/ml and 0.43 mg GAE/g for Rice Formula 2 (after cooking), respectively (Table 2). When Rice Formula 2 was compared (before and after cooking), all levels of γ-oryzanol, α-tocopherol and phenolic substances were found to be different with statistical significance (p<0.05). Biological active compounds are lost during the cooking process at a temperature of 95°C because these substances are unstable under high heat and humidity [30]. Heating temperature and time eliminated substances with antioxidant properties in rice grains and may cause loss while the rice is rinsed before cooking because these substances contain good water solubility properties while α-tocopherol substance levels were no different with statistical significance (p>0.05), possibly because cooking rice at mildly reduced α-tocopherol levels leaves fewer benefits to be gained from significant substances in rice grains.

3.3. Antioxidant Activity

3.3.1. In Vitro

Comparison of the antioxidant activity of Rice Formula 2 with the DPPH method by comparing from concentrations of substances capable of suppressing $DPPH^{\bullet}$ radicals at 50% (IC_{50}) found Rice Formula 2 (before cooking) to have an IC_{50} value of 12.21 mg/ml, which was lower than 37.66 mg/ml for Rice Formula 2 (after cooking) while Rice Formula 2 (before cooking) was able to reduce iron atoms by the FRAP method at 3.27 µmol $FeSO_4$/g, which was better than 3.03 µmol $FeSO_4$/g for Rice Formula 2 (after cooking) (Table 2). When antioxidant activity of Rice Formula 2 (before and after cooking) was compared by the DPPH and FRAP methods, antioxidant activity was found to be different with statistical significance (p<0.05), thus

indicating that Rice Formula 2 (after cooking) had significantly reduced antioxidant capabilities in every method. Antioxidant activity was related to all levels of γ-oryzanol, α-tocopherol, phenolic substances, carotenoid and anthocyanidin in rice grains with reduced values during the cooking process. Therefore, residual antioxidant activity in rice grains after the cooking process may be caused by substances with antioxidant properties not being completely eliminated.

Table 2. Biological active compounds and antioxidant activities of rice formula 2 (before and after cooking)

	Rice Formula 2	
	Before cooking	After cooking
Bioactive compounds		
γ-oryzanol (mg/100g)	42.91±0.98[a]	29.15±1.62[b]
α-tocopherol (µg/ml)	0.30±0.02[a]	0.24±0.02[a]
Total phenolic compounds (	0.85±0.01[a]	0.43±0.01[b]
Antioxidant activity		
DPPH IC_{50} (mg/ml)	12.21±0.06[b]	37.66±3.04[a]
FRAP (µmol $FeSO_4$/g)	3.27±0.00[a]	3.03±0.00[b]

[a,b] The numbers marked with different letters in horizontal order differed with statistical significance (p<0.05).

3.3.2. In Vivo

Table 3. Time in feeding rats with rice formula 2 and glutathione peroxidase activity in rats

Rat feeding times	Glutathione peroxidase activity (unit/ml)			
	Control group	Group 1	Group 2	Group 3
Day 0	0.022±0.00[a]	0.089±0.01[a]	0.081±0.00[d]	0.095±0.00[a]
Day 1	0.021±0.00[a,b]	0.071±0.01[a,b]	0.091±0.00[c,d]	0.078±0.00[b]
Day 3	0.020±0.00[a,b]	0.061±0.01[a,b,c]	0.104±0.00[b,c]	0.072±0.00[b]
Day 7	0.015±0.00[b]	0.054±0.01[b,c]	0.116±0.00[a,b]	0.064±0.00[b,c]
Day 14	0.018±0.00[a,b]	0.040±0.00[c]	0.123±0.00[a]	0.048±0.01[c]
Mean value	0.019±0.00[D]	0.063±0.00[C]	0.103±0.00[A]	0.071±0.00[B]
GPx changes (unit/m	0.004[D]	0.049[A]	0.042[C]	0.047[B]

[a,b,c,d] The numbers marked with different letters in vertical order differed with statistical significance (p<0.05).

[A,B,C,D] The numbers marked with different letters in horizontal order differed with statistical significance (p<0.05).

According to the measurements of rats' GPx levels, GPx levels at Days 0-14 in every group of the experiment were found to differ with statistical significance (p<0.05). On Day 14 of the experiment, the rats in Group 1 and Group 3 were found to have GPx levels reduced to 0.040 and 0.048 unit/ml, respectively (Table 3). The rats in Group 3 were stimulated to have stress by raising the temperature to 38±2°C for 4 hr/day and the rats received 500 mg/kg of polyphenol from extracts of Rice Formula 2, which promoted antioxidant system function. In addition, phenolic compounds had biological the effect of collecting ROS to reduce damage from oxidation stress [31], causing the rats in Group 3 to have reduced GPx levels similar to rats in Group 1 while the rats in Group 2 had higher GPx levels at 0.123 unit/ml, possibly because the rats in Group 2 were stimulated with stress similar to the rats in Group 3. Furthermore, the rats in Group 2 did not receive 500 mg/kg of polyphenol from extracts of Rice Formula 2 combined with the fact that heat was able to raise GPx levels in rats. Nevertheless, animals stressed by body heat will create more oxidants than normal and these molecules will cause oxidation stress [32].

4. Conclusions

The biological active compounds in rice grains have antioxidant properties with roles in preventing heat-related stress. However, heat generated during the cooking process has the effect of reducing nearly half of all biological active compound levels in rice grain while reducing antioxidant activity.

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